Chapter II

MATERIAL AND METHODS

1) MATERIAL :

To study the effects of salts on germination, seeds of Sorghum bicolor (L.) Moench hybrid CSH-9 and variety SPV - 462 have been selected. The seeds of these cultivars were obtained from " Agricultural Research Station, Karad ". The healthy seeds were surface sterilized with 0.1 % $HgCl_2$ solution for 5 minutes. The seeds were then washed thoroughly with distilled water and germinated in sterilized petridishes on filter papers (Whatman No. 1). For salt tolerance studies at germination level, the seeds of CSH-9 and SPV-462 were subjected to various concentrations of NaCl (NaCl : $CaCl_2$, 1:1) and Na_2SO_4. The concentrations used were 25, 50, 100 and 200 mM. Salt treatment was given through Hoagland nutrient medium (1:10 dilution). Control was maintained under non-saline conditions (distilled water). The seeds were then moistened with respective solutions and control with distilled water. The petridishes were kept at room temperature (28 ^{o}C.). Further, doses of respective solutions were added as per the requirments, so that the filter paper was always moist with the respective solution. The treatments were continued upto 120 h (5 days). The germination counts in each treatment were recorded after every 24 h till 96 hours. The emergence of radicle is taken to be the criterion of the germination.

The effect of salt at the 25 to 200 mM concentrations on the germination was observed after every 24 h till 96 hours. The pattern of seedling growth viz. root length, shoot length and fresh wt was studied at the end (after 120 h) of growth.

2) METHODS :

Germination percentage has been studied after every 24 hrs. of growth till 96 hours at the levels from 25 to 200 mM NaCl and Na_2SO_4 concentrations. Seedling growth, carbohydrate content, level of total nitrogen and protein and inorganic constituents from the treated and control seedlings were studied after 120 hrs. of germinations.

A) INORGANIC CONSTITUTENTS :

Inorganic constituents in seedlings were determined from acid digest of oven dried plant material. This method of Toth et al. (1948) was followed for acid degestion process which involved digestion with conc. HNO_3 and perchloric acid.

i) Sodium and Potassium :

Na^+ and K^+ were estimated flame photometrically using standard procedure on flame photometer (model - Elico CL 22 A). For standardization various concentrations of Na^+ and K^+ were prepared ranging from 1 to 10 ppm by diluting stock solutions of NaCl and KCl (100 ppm). Using these standard solutions standard curve for these elements was prepared using flame photometer with specific colour filters. The plant extract was subjected to same procedure. From galvanometer readings the inorganic elements Na^+ and K^+ were estimated using calibration curves of known concentrations of Na^+ and K^+.

ii) Calcium, Iron, Magnesium and Manganese :

These were determined on Atomic Absorption Spectro Photometer.

iii) Phosphorus :

Acid digested extract was also used for estimation of phosphorus by using the method of Durie et al. (1965).

B) ORGANIC CONSTITUENTS :

i) Total Nitrogen and Protein Contents :

From the oven dried material, the total nitrogen content was estimated according to the method of Hawk et al. (1948). Proteins were estimated by multiplying the total nitrogen content by the factor 6.25 (Gilchrist Shirlaw, 1967).

ii) Carbohydrates :

Carbohydrates (total sugars and starch) were estimated, spectrometrically by the method of Nelson (1944). Known quantity of fresh material (seedlings) was homogenized in 80 % ethanol. The extract was filtered through Buchner's funnel using Whatman No. 1 filter paper. The filtrate was used to estimate sugars while the residue was saved for starch estimation.

The filtrate thus obtained was condensed on a water both to about 3-5 ml. This extract was then treated with lead acetate and potassium oxalate (1:1) with constant stirring. To this 30-50 ml. distilled water was added. After that it was filtered through Whatman No. 1 and volume of filterate was measured. This filterate was denoted as "A" for estimation of reducing sugar. From this known volume was hydrolysed with

5 ml. conc. HCl for 30 min. under 15 lb pressure in autoclave. The contents were cooled, neutralized with Na_2CO_3 and filtered. The filtrate was used for the estimation of total sugars.

The residue of alcoholic extract obtained at the beginning was transferred in a conical flask. To it 50 ml. of D. W. and 5 ml. of concentrated HCl were added and hydrolysed at 15 lbs pressure for half an hour and cooled to room temperature. It was then neutralized by Na_2CO_3 and filtered through filter paper Whatman No. 1. The volume of the filtrate was noted. This filtrate containing sugars which was produced as a result of hydrolysis served as a sample for starch estimation.

The requisite quantity of the above filtrates (extracts) was taken separately in 10 ml. marked test tubes. In such other test tubes, different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 ml.) of standard glucose solution (0.1 mg/ml.) were taken. 1 ml. of Somagyi's alkaline copper tartarate reagent (4 g $CuSO_4$, 5 H_2O, 24 g anhydrous Na_2CO_3 ; 16 g Na-K-tartarate, Rochelle salt and 180 g anhydrous Na_2SO_4 dissolved in 1000 ml. distilled water) was added to each test tube. All the reaction mixtures were then subjected to boiling water bath for about 10 min. After cooling to room temperature 1 ml. of arsenomolybdate reagent (25 g ammonium molybdate in 450 ml. water to which were added 21 ml. concentrated H_2SO_4. To this was then added 3 g sodium arsenate, Na_2HASO_4 , 7 H_2O, dissolved in 25 ml. water. All ingradients were mixed well and the

solution was placed in an incubator at 37°C for 48 h. before use) was added to each reaction mixture. The contents of each tube were then diluted to a volume (10 ml.) A blank was prepared by the same way but without sugar solution. After 10 min the absorbance of each reaction mixture was read at 560 nm on spectrophotometer.

By the interpolation from the glucose standard curve, the sugar percentage in the above fractions was determined.